# THE OCCURRENCE OF MELANIN IN THE SEA-URCHIN, DIADEMA ANTILLARUM PHILIPPI*

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## INTRODUCTION

The characteristic dark color of *Diadema antillarum* is chiefly attributable to a black pigment, most of which is contained in large epidermal chromatophores (see Fig. 1). A red pigment is also present, but is easily separable from the black, being readily soluble in ethanol and acetone, whereas the black is not. The nature of the red pigment is unknown and we have not studied its properties.

Correlated with the amount of black pigment is the remarkable sensitivity of the animal to changes in light intensity (1).

The superficial location of the concentrated pigment (Fig. 1), makes it readily accessible, and cytolytic agents in disrupting the extremely delicate epidermis, liberate large quantities of pigment. Indeed, avoiding contamination of the sea water in which the animals are kept, by escaping pigment, is a major problem in the laboratory. Loss of vitality, temperature changes, dilution of the sea water etc., all bring about copious discharge of the black pigment. Large quantities are therefore easily obtained.

*Diadema antillarum* and closely allied species, occur throughout the Antilles, from Florida and Bermuda to Surinam, in the warmer waters of the eastern Atlantic, the Indo-Pacific and from the Gulf of California to the Gulf of Panama (2). Only *Diadema antillarum* has claimed our attention, but it would be surprising indeed if the pigment of allied species did not prove equally suitable and accessible for study. In view of this, and the abundance of these animals, it was considered appropriate to draw the attention of investigators to these facts.

## PROPERTIES OF PIGMENT

The pigment appears in sections of material fixed in Bouin's fluid, as yellow, brown or black granules, or as a reticulum of thin brownish strands with nodal thickenings suggesting somewhat that they may have originated as condensations at the periphery of pre-existing vacuoles or formed bodies of some kind, within the chromatophores. The granules vary in size, most being approximately in the range 0.5 $\mu$ to 6.0 $\mu$ in diameter. The appearance of the pigment in sections of fixed material requires much further study however, and the interpretation of histological preparations is reserved for a separate account.

The chemical and histochemical properties of the crude pigment have been studied by means of simple experiments adequate to characterize the pigment as a melanin (3). Especially characteristic are the insolubility, bleaching when

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subjected to the action of strong oxidizing agents, and the capacity to reduce directly, ammoniacal solutions of silver nitrate.

Solubility tests were performed on the crude black pigment obtained by cytolysis, as a result of placing the whole animal, or portions, in fresh water; or by grinding in a mortar, fragments of the cleaned eviscerated test (shell) with its covering tissues and spines, followed by separation of the pigment by centrifuging or filtering.

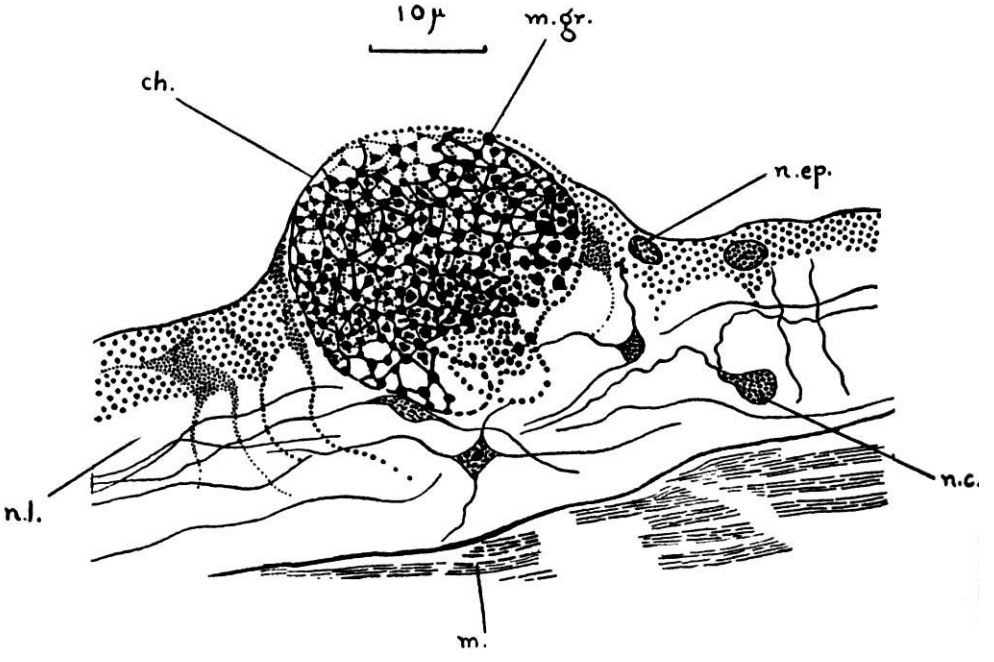


FIG. 1. Chromatophore in epidermis of *Diadema antillarum*. Fixed Bouin's fluid, stained Mallory's triple stain.

| | |
|---|---|
| ch. | chromatophore |
| m. | muscle layer |
| m.gr. | melanin granule |
| n.c. | nerve cell |
| n.ep. | nucleus of epidermal cell |
| n.l. | nervous layer |

The crude paste of pigment thus obtained was dried and extracted with solvents for several hours or days and the results may be summarized as shown in Table 1.

The effect of oxidizing agents on a paste, or suspension, of the crude pigment was ascertained. Hydrogen peroxide, bromine water, chlorine, chromic acid, potassium permanganate and oxalic mixtures, and chloro-dioxy-acetic acid, all decolorized the pigment. Such action is, of course, in harmony with that of the nitric acid noted above.

By way of confirmation, the effect of some of the above oxidizing agents on

the pigment in fixed chromatophores was ascertained, sections of a young urchin being subjected to their action for 24 hours.

Urchins were fixed for 20 hours in Bouin's fluid, decalcified by sodium hexametaphosphate, embedded in wax and sectioned at 10 $\mu$. The sections were then treated with chlorine, bromine, or potassium permanganate, using the methods of Mayer, Mawas and Alfieri (see Lison 1936 (3)).

In each instance, the pigment in the chromatophores was seen to be completely decolorized.

TABLE 1

| SOLVENT | RESULT | REMARKS |
|---|---|---|
| water | slightly soluble | |
| 90% ethanol | insoluble | |
| benzene | insoluble | |
| petrol ether | insoluble | |
| carbon disulphide | insoluble | |
| acetone | insoluble | |
| pyridine | slightly soluble | Pigment which dissolved seemed somewhat different from insoluble portion, being reddish, whereas the latter was black. Reddish fraction was readily soluble in pyridine. |
| ethylene chlorohydrin | insoluble | None dissolved after 4 days extraction with solvent. |
| $\frac{N}{1}$ hydrochloric acid | slightly soluble | Formed a reddish solution, yielding a purple residue on evaporation to dryness. The residue was insoluble in distilled water, but readily in $\frac{N}{1}$ HCl forming a reddish solution. Solubility markedly increased on warming. |
| concentrated hydrochloric acid | soluble | Formed a reddish solution. |
| $\frac{N}{1}$ nitric acid | pigment bleached | Changed rapidly from black, through brown, to orange yellow. |
| concentrated nitric acid | pigment bleached | Changed rapidly to red, then decolourized. |

Sections prepared by the same method were subjected to Masson's technic for the argentaffin reaction (3). It was observed that the skin chromatophores readily reduce ammoniacal silver nitrate.

Thus the black pigment located in the skin chromatophores shows the characteristic properties of melanin.

It is pertinent that a similar pigment can be formed in the amoebocytes of the coelomic fluid. As has already been reported (4), the coelomic fluid, when exposed to air, throws down a clot formed of aggregated and disintegrated amoebocytes.

On continued exposure, the clot forms a brown pigment, a process which is associated with certain spheroidal inclusions of the amoebocytes, atmospheric oxidation and phenolase activity. The formation and darkening of the clot recalls similar processes seen to occur in the coelomic fluid of the holothurian *Thyone briareus* (Lesueur), (Millott 1950 (5)). The behavior of the pigment formed by the clot, toward solvents and oxidizing agents, corresponds closely to that of the pigment in the skin chromatophores; moreover, the amoebocytes of the clot show a marked argentaffin reaction.

These facts are significant in indicating a possible site and mode of origin of the skin pigment; they do not however, justify making claims stronger than this, and the relationship of the pigment found in the skin to that formed in the amoebocytes of the coelomic fluid, requires further elucidation.

The foregoing chemical and histochemical properties are sufficient to characterize the black or brown pigment of the skin chromatophores, and that developed in the coelomic amoebocytes on exposure to air, as a melanin within the limits necessarily imposed by the present inadequate state of our knowledge of these pigments. It should be noted however, that its properties differ somewhat from those ascribed to melanins in other animals by various investigators.

Thus Bloch and Schaaf (6), have described melanin as soluble in alcohol, whereas the pigment we have studied, is insoluble, at least in 90% ethanol. Lea (7), has described a melanin from the ink sac of *Sepia officinalis*, as soluble in ethylene chlorohydrin, yet that from *Diadema* is insoluble. Again, the melanins from a malignant melanoma and negro skin are described by Taft (8), as insoluble in water and pyridine, as well as in alcohol and ethylene chlorohydrin, but the pigment from *Diadema* is slightly soluble in the first two. The variation is not surprising, as the pigments usually studied may well have been altered during extraction, and that obtained by us, was almost certainly impure as well. Also, there are indications that the melanins described by various investigators are not the same substance, but differ perhaps, in constitution, degree of polymerisation (9), and in the protein with which they may be conjugated.

It is of interest to record the occurrence of a melanin in echinoids, as affording further evidence of the wide distribution of these pigments in invertebrates (see Fox (10)), and especially in view of their reported occurrence in ophiuroids and holothurians among the echinoderms (5, 11).

The occurrence of a melanin in quantity, located superficially and therefore readily accessible, should attract the interest of dermatologists seeking to study melanin and its formation in a wider sphere.

As has been mentioned above, the pigment occurs largely in chromatophores, but it is emphasized that this term is used in the sense in which it is employed by zoologists, namely, to designate a pigment bearing cell. The term as used here, does not carry the special significance given to it by Masson (12), who confines its application to cells which contain pigment without having produced it. It is not known how the pigment of these cells originates, and we are now undertaking further investigations along these lines. It may be significant that, as mentioned above, the coelomic amoebocytes which can form a pigment closely

similar to the chromatophore melanin, contain phenolases, and further, their pigment forming activity is increased by ultra-violet light and sometimes inhibited by the mono-benzyl ether of hydroquinone ("agarite alba") (4).

SUMMARY

The skin chromatophores and coelomic amoebocytes in *Diadema antillarum Philippi* are shown to contain a pigment having the properties of melanin. The pigment is readily accessible for dermatological studies. The properties of the pigment are described and discussed in relation to those of other animals.

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